REMARKS

Claims 1, 3-4, 6, 8, 12-16, 19, 21, 23-24, 26, 29, and 139-161 are pending in the present application. Claims 5, 7, 9-11, 17-18, 22, 25, 27-28, 34-35, 41-42, 45-111, 113, 115-117, 126-127, 129-130 and 134 have previously been cancelled without prejudice or disclaimer. Claims 2, 6, 20, 30-33, 36-40, 43-44, 112, 114, 118-125, 128, 131-133, and 135-138 are cancelled without prejudice or disclaimer.

This Amendment and Response is filed supplemental to the response filed on December 2, 2009 which was filed responsive to the non-final Official Action dated April 15, 2009. The December 2, 2009 response is incorporated herein in its entirety.

Claims 1, 8, and 21 have been amended, and claims 2, 6, 20, 30-33, 36-40, 43-44, 112, 114, 118-125, 128, 131-133, and 135-138 have been cancelled without prejudice or disclaimer. Additionally, new claims 139-161 have been added. Support for these amendments appears throughout the specification and claims as originally filed. No new matter has been added. Applicants, by amending any claims herein and/or canceling any claims herein, make no admission as to the validity of any rejection made by the Examiner against any of these claims. Applicants reserve the right to reassert any of the claims canceled herein or the original claim scope of any claim amended herein, in a continuing application.

Applicants thank the Examiner for conducting an interview with the undersigned attorney on December 8, 2009. During the interview the rejections of

record were discussed and Applicants agreed to consider amending the claims and submitting evidence of unexpected results or commercial success. Accordingly, Applicant's have amended the claims as presented above. Further, the discussed evidence of unexpected results and commercial results is submitted and discussed herein below.

Claims 1 and 21 have been amended to recite: (i) that the minoxidil or minoxidil salt is the "sole hair-growing active present in the composition"; (ii) to delete "an oil component" and "a propellant" and optional excipients; (iii) to require that the pharmaceutical composition is actuated with a propellant to form a foam or mousse; (iv) to specify that "the minoxidil or salt thereof is not encapsulated"; and (v) to specify "wherein the apparent pH of the final product is in the range of from approximately 5.0 to 7.0." Support for amended claims 1 and 21, and new claims 139-161, appears throughout the specification and claims as originally filed. Further, claim 8 has been amended to change the claim dependency in view of the cancellation of claim 2. No new matter has been added.

In view of the following, further and favorable consideration is respectfully requested.

I. At page 3 of the final Official Action, the Examiner has maintained the rejection of claims 1-4, 6, 8, 12, 13, 15-16, 19-21, 23-24, 26, 29, 112, 114, 118-125, 128, 131-132 and 135-138 under 35 USC § 103 (a), as being unpatentable over Peck et al. in view of Weiner et al. or Yu et al., respectively.

The Examiner asserts that "It would have been obvious to the skilled artisan

to combine the teachings of Peck et al. and Weiner and utilize the instant minoxidil acid salt" because Weiner teaches that this addition yields a hydrophilic compound that allows for better penetration into the hair follicles.

With regard to claims 1 and 112, the Examiner also asserts that while the claims were amended to recite the transition language "consisting of," they were also amended to optionally include one or more excipients that would allow for the inclusion of lipid vesicles which would be considered an excipient, i.e., a penetration agent.

In view of the following, the secondary considerations discussed below, and supplemental to the response filed on December 2, 2009, this rejection is respectfully traversed.

Claims 3-4, 8, 12-13, 15-16, 19, 20, 26, and 29 are directly or indirectly dependent on independent claim 1. Claims 23-24 are each directly dependent on independent claim 21. Claims 2, 6, 20, 112, 114, 118-125, 128, 131-132, and 135-138 have been canceled. Present claims 1 and 21 have been amended, in part, to specify that "the minoxidil or salt thereof is not encapsulated". More specifically, claims 1 and 21 clearly exclude the inclusion of minoxidil in a lipid vesicle. In contrast, a lipid vesicle is clearly required by Weiner et al. Weiner et al., in the "Summary of the Invention", states that the "invention" is "based, in part, on the discovery that making a material...and encapsulating the drug in a lipid vesicle, can improve delivery. This is particularly pertinent to the delivery of minoxidil." *See*

also, Weiner et al. in the claims.

Weiner et al. ***teach away*** from preparing and/or administering an unencapsulated minoxidil acid salt formulation. The skilled artisan in view of Weiner et al. would have had no motivation to prepare a formulation by employing a minoxidil salt or by reacting minoxidil with an acid to form a minoxidil salt, ***absent encapsulation*** of the minoxidil salt in a lipid vesicle because Weiner et al. describe that an unencapsulated minoxidil salt exhibits extremely poor penetration. Again, the present claims specifically do not encompass encapsulation of minoxidil in lipid vesicles.

In view of the foregoing, it is submitted that nothing in the applied references, taken alone or together, render the presently claimed subject matter obvious within the meaning of 35 USC § 103. Accordingly, the Examiner is respectfully requested to withdraw this rejection of pending claims 1, 3-4, 6, 8, 12, 13, 15-16, 19, 21, 23-24, 26, and 29.

II. At page 11 of the Official Action, the Examiner has maintained the rejection of claims 14, 30-33, 36-40, 43-44 and 133 under 35 USC § 103 (a), as being unpatentable over Peck et al. in view of Weiner et al. or Yu et al., respectively, and further in view of Uchikawa et al.

The Examiner asserts that it would have been obvious to the skilled artisan "to combine the teachings of the above references and substitute the exemplified propylene glycol with the instantly claimed glycerol and arrive at the instant invention" because "Uchikawa et al. teach both propylene glycol and glycerol are

polyhydric alcohols conventionally used in the art."

In view of the following, the secondary considerations discussed below, and supplemental to the response filed on December 2, 2009, this rejection is respectfully traversed.

Claim 14 is indirectly dependent on independent claim 1. Claims 30-33, 36-40, 43-44, and 133 have been cancelled. Claim 1 has been amended to specify that "the minoxidil or salt thereof is not encapsulated". Claim 30 specifically excludes encapsulation of minoxidil in a lipid vesicle.

As discussed above, Weiner et al. ***teach away*** from preparing and/or administering an unencapsulated minoxidil acid salt formulation. The skilled artisan in view of Weiner et al. would have had no motivation to prepare a formulation by employing a minoxidil salt or by reacting minoxidil with an acid to form a minoxidil salt, ***absent encapsulation*** of the minoxidil salt in a lipid vesicle because Weiner et al. describe that an unencapsulated minoxidil salt exhibits extremely poor penetration. Again, the present claims specifically do not encompass encapsulation of minoxidil in lipid vesicles.

In view of the foregoing, it is submitted that nothing in the applied references, taken alone or together, render the presently claimed subject matter obvious within the meaning of 35 USC § 103. Accordingly, the Examiner is respectfully requested to withdraw this rejection of pending claim 14.

III. At page 12 of the Official Action, the Examiner has maintained the rejection of claims 1-4, 6, 8, 12-13, 15-16, 19-21, 23-24, 26, 112, 118-125, 128, 131-132 and 135-138 under 35 USC § 103 as being unpatentable over JP 07-048230 in view of Weiner et al. or Yu et al. and further in view of Caldini et al.

The Examiner asserts that it would have been obvious to the skilled artisan to combine the teachings of JP 07-048230 ("'230") and Weiner et al. or Yu et al. and utilize the instant minoxidil acid salt. The Examiner further asserts that it would have been obvious to the skilled artisan to combine the teachings of the references and utilize benzyl alcohol in the solvent system because Caldini et al. teach that the use of benzyl alcohol improves transcutaneous and transfollicular absorption of active agents.

In view of the following, the secondary considerations discussed below, and supplemental to the response filed on December 2, 2009, this rejection is respectfully traversed.

As discussed above in sections I and II, incorporated herein by reference in their entirety, present claim 1 recites the transition language "consisting of." Claim 21 recites providing a pharmaceutical composition "consisting of." This transition language excludes components other than those expressly recited. In addition, claims 1 and 21 have been amended to specify that "the minoxidil or salt thereof is not encapsulated". Accordingly, claims 1 and 21 clearly exclude encapsulation of minoxidil in lipid vesicles. *See* sections I and II herein. Further, claims 2, 6, 20, 112, 118-125, 128, 131-132, and 135-138 have been canceled.

As discussed above, Weiner et al. ***teach away*** from preparing and/or administering an unencapsulated minoxidil acid salt formulation. The skilled artisan in view of Weiner et al. would have had no motivation to prepare a formulation by employing a minoxidil salt or by reacting minoxidil with an acid to form a minoxidil salt, ***absent encapsulation*** of the minoxidil salt in a lipid vesicle because Weiner et al. describe that an unencapsulated minoxidil salt exhibits extremely poor penetration. Again, the present claims specifically do not encompass encapsulation of minoxidil in lipid vesicles.

Further, as discussed above, the skilled artisan would have had no motivation to prepare a formulation employing a minoxidil salt or prepare a formulation by reacting minoxidil with an acid to form a minoxidil salt, because Weiner et al. describe that commercial Rogaine® (that is encapsulated) exhibits significantly greater penetration than exhibited by an unencapsulated minoxidil salt formulation and thus ***teach away*** from the present claims. Again, the present claims do not encompass encapsulation of minoxidil in lipid vesicles.

In view of the foregoing, it is submitted that nothing in any of JP 07-048230, Weiner et al., Yu et al., and Caldini et al., taken alone or together, suggests the presently claimed subject matter within the meaning of 35 USC § 103. Accordingly, the Examiner is respectfully requested to withdraw this rejection of pending claims 1, 3-4, 8, 12-13, 15-16, 19, 21, 23-24, and 26.

IV. At page 15 of the Official Action, the Examiner has maintained the rejection of claims 1-4, 6, 8, 12-13, 15-16, 19-21, 23-24 and 26-29 under 35 USC § 103 (a), as being unpatentable over Navarro et al. in view of Weiner et al.

The Examiner asserts that it would have been obvious to the skilled artisan "to combine the teachings of Navarro et al. and Weiner and substitute Navarro's cyclodextrin with the instant acid to convert minoxidil into a salt" because "Weiner teaches that by converting minoxidil to a hydrophilic compound, it penetrates the skin."

In view of the following, the secondary considerations discussed below, and supplemental to the response filed on December 2, 2009, this rejection is respectfully traversed.

Responsive to Applicants arguments, the Examiner asserts that claims 1 and 21 allow for the inclusion of a lipid vesicle or a cyclodextrin carrier because they recite an optional excipient which can be a lipid vesicle or a cyclodextrin carrier. More specifically, the Examiner asserts that "the cyclodextrin carrier of Weiner [Navarro] reads on a stabilizer. As evidenced by Moldenhauer et al...compositions containing complexes of gamma-cyclodextrin and retinol or retinol derivatives have unexpected stability. Thus, Moldenhauer et al. support the examiner's argument that cyclodextrin reads on a stabilizer. As such,...applicant's claims do not exclude Weiner's [Navarro's] cyclodextrin carrier." The Examiner also asserts that the use of cyclodextrin salt addition provides substantial penetration through the hair follicle

second only to the use of a minoxidil acid salt addition. *See* the Official Action of April 15, 2009 at page 15, paragraph 4 and page 17 paragraphs 3 and 4.

Applicants strongly disagree with the Examiner's assertion that the present claims encompass a cyclodextrin carrier. None of claims 1and 21 recite an optional excipient that is a cyclodextrin carrier. The recited optional excipients neither include a cyclodextrin carrier nor allow for the inclusion of a cyclodextrin carrier.

As discussed above in sections I and II, incorporated herein by reference in their entirety, present claim 1 recites the transition language "consisting of." Claim 21 recites providing a pharmaceutical composition "consisting of." This transition language excludes components other than those expressly recited. In addition, claims 1 and 21 have been amended to specify that "the minoxidil or salt thereof is not encapsulated"; and to specify "wherein the apparent pH of the final product is in the range of from approximately 5.0 to 7.0." Accordingly, claims 1 and 21 clearly exclude encapsulation of minoxidil in a cyclodextrin carrier. Please *see* sections I and II herein.

Navarro et al. describe encapsulating minoxidil in a cyclodextrin carrier, wherein cyclodextrin functions as a "host" molecule to trap the minoxidil "guest" molecule inside the ring. Navarro et al. describe the use of cyclodextrin in order to assist in the solubilization of minoxidil while avoiding high amounts of propylene glycol. Navarro et al. state the following:

> [t]he amount of γ-cyclodextrin present in the composition for hair is such that it permits a substantial reduction in the amount of solvent

> for minoxidil which would normally need to be added to achieve a comparable solubility of minoxidil in the absence of the aforementioned cyclodextrin. (Page 3, lines 9-13 of the English translation).

From the foregoing, it is clear that cyclodextrin is an essential element of Navarro et al. because it must be combined with minoxidil in order to impart improved solubility properties to minoxidil, thereby reducing the amount of solvent such as propylene glycol needed in the formulation. However, the present claims ***exclude*** cyclodextrin. As such, the encapsulating technique of Navarro et al. is distinguished. Please *see* sections I and II herein.

In further support of the foregoing, the Examiner's attention is directed to the Declaration submitted previously in corresponding patent application no. 10/124,197 now US Patent No. 6,946,120, on June 18, 2004. The Declaration under 37 CFR §1.132 by Albert Zorko Abram ("the Abram Declaration"), was filed in response to a rejection of the then-pending claims under 35 USC § 103 as obvious in view of the disclosures of Navarro in view of Weiner et al. and further in view of Leitch.

In the Declaration, Mr. Abram declares in paragraph 9 that supplementing the teaching of Navarro with the teaching of Weiner et al. ***would destroy the intended purpose of the Navarro composition***. Mr. Abram declares in paragraph 10 that the role of cyclodextrin in Navarro is to function as a host molecule to trap the minoxidil "guest" molecule inside the ring and that it is the minoxidil-cyclodextrin "host-guest" complex that imparts improved solubility properties, as compared to a similar minoxidil composition not having cyclodextrin. Mr. Abram declares that it is

recognized that ***cyclodextrins are unstable in acidic conditions*** and that subjecting cyclodextrins to acidic conditions will result in the degradation of the cyclodextrins into its individual glucose units. The present claims require that the pH of the final product is in the range of from approximately 5.0 to 7.0, i.e., ***acidic***.

In view of the foregoing, it is submitted that nothing in Navarro et al. and Weiner et al., taken alone or together, renders the presently claimed subject matter obvious within the meaning of 35 USC § 103. Accordingly, the Examiner is respectfully requested to withdraw this rejection of pending claims 1, 3-4, 8, 12-13, 15-16, 19, 21, 23-24, 26 and 29.

V. At page 18 of the Official Action, the Examiner has maintained the rejection of claims 14, 30-33, 36-40, 43-44, 112, 118-125, 128 and 131-138 under 35 USC § 103 (a), as being unpatentable over Navarro et al. in view of Weiner et al. and further in view of Wong et al.

The Examiner asserts that it would have been obvious to the skilled artisan to combine the teachings of the references and further utilize a propellant because "Wong et al. teach that a propellant allows a solution to aerosolize." The Examiner further asserts that "it would have been obvious to use either propylene glycol or glycerol and arrive at the instant invention."

In view of the following, the secondary considerations discussed below, and supplemental to the response filed on December 2, 2009, this rejection is respectfully traversed.

Claim 14 is indirectly dependent on independent claim 1. Claims 30-33, 36-

40, 43-44, 112, 118-125, 128 and 131-138 have been cancelled. Claim 1 specifically excludes encapsulation of minoxidil in a lipid vesicle and/or in a cyclodextrin carrier as discussed above in section IV, incorporated herein by reference in its entirety.

As discussed above, present claim 1 recites the transition language "consisting of." This transition language excludes components other than those expressly recited. In addition, claim 1 has been amended to specify that "the minoxidil or salt thereof is not encapsulated". Accordingly, claims 1 clearly excludes encapsulation of minoxidil in lipid vesicles and/or in a cyclodextrin carrier.

In view of the foregoing, it is submitted that nothing in Navarro et al., Weiner et al., and Wong et al., taken alone or together, renders the presently claimed subject matter obvious within the meaning of 35 USC § 103. Accordingly, the Examiner is respectfully requested to withdraw this rejection of pending claim 14.

VI. At page 20 of the Official Action, claims 30-31, 36-40, 43-44, 112, 114, 118-125, 128, 131-132 and 135-138 have been rejected under 35 USC § 103 (a), as being unpatentable over Navarro et al. in view of Weiner et al. and further in view of Peck et al.

The Examiner asserts that it would have been obvious to the skilled artisan "to combine the teaching of the above references and further utilize the instant excipients."

However, all of claims 30-31, 36-40, 43-44, 112, 114, 118-125, 128, 131-132, and 135-138 are canceled herein. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

VII. At page 21 of the Official Action, the Examiner has rejected claims 1-4, 6, 8, 12-13, 15-16, 19-21, 23-24, 26 and 29 under 35 USC § 103 as being unpatentable over Bazzano in view of Weiner or Yu et al.

The Examiner asserts that it would have been obvious to the skilled artisan to combine the teachings of Bazzano and Weiner et al. and utilize the instant minoxidil acid salt. Alternatively, the Examiner asserts that it would have been obvious to combine the teachings of Bazzano and Yu et al. and utilize the instant acid because Yu et al. teach adding lactic acid dissolves minoxidil providing better penetration of minoxidil into the hair follicle.

In view of the following, the secondary considerations discussed below, and supplemental to the response filed on December 2, 2009, this rejection is respectfully traversed.

Bazzano is directed to a synergistic combination of a retinoid and a minoxidil compound for topical application to the skin, hair and/or hair follicles of a mammal. Bazzano clearly requires that the combination include a retinoid. Bazzano does not teach or suggest a combination absent a retinoid as a second active ingredient, as it describes a ***synergistic*** combination. *See* the abstract; col. 1, lines 20-30; and col. 3, lines 59-68.

The Examiner asserts that the present claims still allow for the inclusion of a lipid vesicle or a cyclodextrin carrier because they could be considered excipients.

The Examiner further asserts that the retinoic acid required by Bazzano reads on the presently claimed penetration enhancer.

Claim 1 recites the transition language "consisting of." Claim 21 recites providing a pharmaceutical composition "consisting of." This transition language excludes components other than those expressly recited. More specifically, the transition language "consisting of" excludes encapsulation of minoxidil in lipid vesicles as well as a cyclodextrin carrier, and a retinoid. In addition, claims 1 and 21 have been amended to recite: (i) that the minoxidil or minoxidil salt is the "sole hair-growing active present in the composition"; (ii) to specify that the pharmaceutical composition is actuated with a propellant to form a foam or mousse; (iii) to delete "an oil component"; (iv) to specify that "the minoxidil or salt thereof is not encapsulated"; and (v) to specify "wherein the apparent pH of the final product is in the range of from approximately 5.0 to 7.0." Accordingly, claims 1 and 21 clearly specifically exclude encapsulation of minoxidil in a lipid vesicle and a cyclodextrin carrier.

Applicants strongly disagree with the Examiner's assertion. None of claims 1 or 21 recite an optional excipient that is a retinoid. The recited optional excipients neither include a retinoid nor allow for the inclusion of a retinoid. Further, the retinoid is described as an active agent in Bazzano, ***not*** an excipient. In contrast, the recitation, in present claims 1 and 21, that the minoxidil or minoxidil salt is the "sole hair-growing active present in the composition" clearly excludes the retinoid

active agent of Bazzano et al.

In view of the foregoing, it is submitted that nothing in any of the applied references, taken alone or together, suggest the subject matter of claims 1, 3-4, 6, 8, 12-13, 15-16, 19, 21, 23-24, 26 and 29 within the meaning of 35 USC § 103. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

VIII. Secondary Considerations

With regard to each of the above discussed 103 rejections, assuming *arguendo* that a prima facie case of obviousness has been established, Applicant's submit that the below discussed evidence is sufficient to rebut any case of prima facie obviousness.

With regard to rebuttal of a prima facie case of obviousness, MPEP §2145 recites the following:

> Rebuttal evidence may include evidence of "secondary considerations," such as "commercial success, long felt but unsolved needs, [and] failure of others." *Graham v. John Deere Co.*, 383 U.S. at 17, 148 USPQ at 467. See also, e.g., *In re Piasecki*, 745 F.2d 1468, 1473, 223 USPQ 785, 788 (Fed. Cir. 1984) (commercial success). Rebuttal evidence may also include evidence that the claimed invention yields unexpectedly improved properties or properties not present in the prior art. Rebuttal evidence may consist of a showing that the claimed compound possesses unexpected properties. *Dillon*, 919 F.2d at 692-93, 16 USPQ2d at 1901. A showing of unexpected results must be based on evidence, not argument or speculation. *In re Mayne*, 104 F.3d 1339, 1343-44, 41 USPQ2d 1451, 1455-56 (Fed. Cir. 1997) ... A conclusion of obviousness requires that the reference(s) relied upon be enabling in that it put the public in possession of the claimed invention....

Consideration of rebuttal evidence and arguments requires Office personnel to weigh the proffered evidence and arguments. Office personnel should avoid giving evidence no weight, except in rare circumstances. *Id.* See also *In re Alton*, 76 F.3d 1168, 1174-75, 37 USPQ2d 1578, 1582-83 (Fed. Cir. 1996). However, to be entitled to substantial weight, the applicant should establish a nexus between the rebuttal evidence and the claimed invention, i.e., objective evidence of nonobviousness must be attributable to the claimed invention. The Federal Circuit has acknowledged that applicant bears the burden of establishing nexus....

When considering whether proffered evidence is commensurate in scope with the claimed invention, Office personnel should not require the applicant to show unexpected results over the entire range of properties possessed by a chemical compound or composition. See, e.g., *In re Chupp*, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987). Evidence that the compound or composition possesses superior and unexpected properties in one of a spectrum of common properties can be sufficient to rebut a *prima facie* case of obviousness. *Id....*

For example, a showing of unexpected results for a single member of a claimed subgenus, or a narrow portion of a claimed range would be sufficient to rebut a *prima facie* case of obviousness if a skilled artisan "could ascertain a trend in the exemplified data that would allow him to reasonably extend the probative value thereof." *In re Clemens*, 622 F.2d 1029, 1036, 206 USPQ 289, 296 (CCPA 1980) (Evidence of the unobviousness of a broad range can be proven by a narrower range when one skilled in the art could ascertain a trend that would allow him to reasonably extend the probative value thereof.). But see, *Grasselli*, 713 F.2d at 743, 218 USPQ at 778 ... Accordingly, each case should be evaluated individually based on the totality of the circumstances....

Evidence pertaining to secondary considerations must be taken into account whenever present; ...See, e.g., *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1372, 82 USPQ2d 1321, 1339 (Fed. Cir. 2007) ... Office personnel should not evaluate rebuttal evidence for its "knockdown" value against the *prima facie* case, *Piasecki,* 745 F.2d at 1473, 223 USPQ at 788, or summarily dismiss it as not compelling or insufficient. If the evidence is deemed insufficient to rebut the *prima*

facie case of obviousness, Office personnel should specifically set forth the facts and reasoning that justify this conclusion.

A. Evidence of Commercial Success

Attached is sales data (Appendix **A**) from Nielsen, a publicly available commercial search service, which shows data from 2005 through 2009 for minoxidil topical solution ("MTS") which contains 5% minoxidil, water, alcohol and propylene glycol, a further minoxidil solution ("other solution") and the presently claimed minoxidil topical foam which contains 5% minoxidil in a hydroalcoholic, propylene glycol-free, unencapsulated formula ("MTF").

As can be seen from the Nielsen data, the numbers clearly indicate a steady increase in the sales of the present MTF and a steady decrease in the sales of MTS. Specifically, the presently claimed MTF was introduced in 2006 and enjoyed 384% growth in 2007, while MTF experienced -27% growth. In 2008, MTF enjoyed 33% growth, while MTF experienced -25% growth. Lastly in 2009, MTF enjoyed 10% growth, while MTF experienced -70% growth.

In view of the foregoing, Applicant's submit that any prima facie case of obviousness is rebutted by the Nielsen data submitted herewith illustrating the commercial success of the claimed minoxidil compositions actuated to form a mousse or foam. Accordingly, the Examiner is respectfully requested to withdraw each of the above-discussed obviousness rejections of record.

B. Evidence of Unexpected Results

Applicant's submit that the claimed minoxidil compositions, i.e., MTF, exhibit

unexpectedly improved properties as compared to MTS, as evidenced by Olsen, et al. "*A multicenter, randomized, placebo-controlled, double-blind clinical trial of a novel formulation of 5% minoxidil topical foam versus placebo in the treatment of androgenetic alopecia in men*," American Academy of Dermatology, Inc, vol. 57, no. 5, pp. 767-774 (April 10, 2007). (Appendix **B**)

Olsen et al. describe, on page 767, a minoxidil topical solution ("MTS") which contains 5% minoxidil, water, alcohol and propylene glycol, and the presently claimed minoxidil topical composition actuated to form a foam or mousse which contains 5% minoxidil in a hydroalcoholic, propylene glycol-free, unencapsulated formula ("MTF").

At page 768, col. 1, Olsen et al. describe two preclinical studies that evaluated the comparative efficacy of the MTF and MTS vehicles, as follows:

> In the hamster ear model for assessing follicular targeting, the 5% MTF showed increased uptake of minoxidil over the 5% MTS at both 1 and 2 hours of application.[5] [*See* Ewing et al., "*Update of minoxidil from a new foam formulation devoid of propylene glycol to hamster ear hair follicles.*" J. Invest. Dermatol. 2005; Abstract 606:A101 (Appendix **C**)] A direct comparison of the efficacy of 5% MTF and 5% MTS was performed in the stump-tailed macque,[6] [*See* Rundegren et al., "*Hair growth efficacy assessment of a new topical minoxidil foam formulation in the stumptail macaque*," J. Invest. Dermatol. 2005; Abstract 587:A98 (Appendix **D**)] an animal model for AGA [androgenetic alopecia] in humans.[7,8] Six macaques were treated topically with either water, 5% MTS or 5% MTF once daily for sequential 4-month trial periods with 3-month washout periods between treatment groups.[6] Change in target area hair weight between baseline and month 4 of each treatment period was the primary end point. The macaques had an increase in hair weight of 12.40 mg (8.23-26.00 mg) on 5% MTF compared with an increase in hair weight of 9.27 mg (4.96-17.53 mg) on 5% MTS.

> Two human pharmacokinetic (PK) studies were completed.[9] [Rogaine In-Home Use Test, Final Report, December 2003. Data on File, Pfizer, Inc.] One study compared 5% MTS to 5% MTF and showed that the systemic absorption of the 5% MTF with twice-daily application of 1 g (one-half capful) in men was about half of that observed with 5% MTS with twice-daily application of 1 cc (both 100 mg daily of minoxidil); this was evidenced by the area under the curve of serum minoxidil concentration and maximum serum minoxidil concentration. The second study was an exaggerated-use PK study in men that demonstrated that application of up to 3 g of the 5% MTF or 300 mg of minoxidil (3 times the recommended dose) twice daily resulted in systemic levels that were well below the 21 µg/mL threshold for cardiac-related events and, thus, within acceptable safety margin. Consumer use studies[9] showed that the minoxidil foam vehicle was rated significantly higher on several aesthetic attributes compared with minoxidil solution, including ease of application, lack of dripping, quick absorption and drying, and ability to fit easily into a daily routine.

As can be seen from the above, the presently claimed minoxidil composition, i.e., MTF, when compared to propylene glycol-containing MTS, exhibited significantly improved efficacy in vivo in the stump-tailed macaque. The stump-tailed macaque is an accepted model for androgenetic alopecia. Specifically, hair growth, as measured by an increase in hair weight, increased by 12.40 mg in the MTF treated macaque group and by 9.27 mg in the MTS treated macaque group. Thus, the MTF treated group showed a ***34 % improvement*** over the MTS treated group. *See* Appendix D.

In the hamster ear model for assessing follicular targeting, the presently claimed minoxidil composition, i.e., 5% MTF, showed increased uptake of minoxidil over the 5% MTS at both 1 and 2 hours of application. *See* Appendix C.

In addition, as set forth above, two human pharmacokinetic (PK) studies were completed. One study compared 5% MTS to the present 5% MTF and showed that the systemic absorption of the 5% MTF with twice-daily application of 1 g (one-half capful) in men was about half of that observed with 5% MTS with twice-daily application of 1 cc (both 100 mg daily of minoxidil); this was evidenced by the area under the curve of serum minoxidil concentration and maximum serum minoxidil concentration.

Lastly, consumer use studies showed that the minoxidil foam vehicle was rated significantly higher on several aesthetic attributes compared with minoxidil solution, including ease of application, lack of dripping, quick absorption and drying, and ability to fit easily into a daily routine.

In view of the foregoing, Applicant's submit that any prima facie case of obviousness is rebutted by the above-discussed evidence of the unexpectedly superior properties of the claimed minoxidil composition, i.e., minoxidil topical composition actuated to form a foam or mousse, as compared to propylene glycol-containing minoxidil topical solution. Accordingly, the Examiner is respectfully requested to withdraw each of the above-discussed obviousness rejections of record.

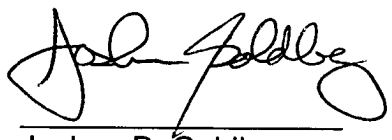
CONCLUSION

Applicants assert that the claims are in condition for immediate allowance and early notice to that effect is earnestly solicited. Should the Examiner deem that any further action by Applicants' undersigned representative is desirable and/or necessary, the Examiner is invited to telephone the undersigned at the number set forth below.

In the event this paper is not timely filed, Applicants petition for an appropriate extension of time. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 14-0112.

Respectfully submitted,

THE NATH LAW GROUP



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APPENDIX A

Rogaine (5% Monoxidil)

	Sales $ by Year				
	'05	'06	'07	'08	'09
Solution	$18,516,742	$16,538,028	$12,153,607	$9,060,279	$2,759,815
Foam	$0	$3,951,998	$19,135,285	$25,439,857	$27,981,738
Other Solution	$0	$0	$0	$2,750,363	$7,563,714
TOTAL	**$18,516,742**	**$20,490,026**	**$31,288,892**	**$37,250,498**	**$38,305,267**

	Growth %			
	'06	'07	'08	'09
Solution	-11%	-27%	-25%	-70%
Foam		384%	33%	10%
Other Solution				175%
TOTAL	**11%**	**53%**	**19%**	**3%**

	Average Price				
	'05	'06	'07	'08	'09
Solution	$36	$36	$37	$39	$31
Foam		$33	$36	$39	$40
Other Solution				$47	$48
TOTAL	**$36**	**$35**	**$37**	**$40**	**$41**

	Units $ by Year				
	'05	'06	'07	'08	'09
Solution	512,077	456,234	326,880	232,460	89,716
Foam	0	121,516	528,055	646,933	692,743
Other Solution	0	0	0	58,070	157,537
TOTAL	**512,077**	**577,750**	**854,935**	**937,463**	**939,995**

	Growth %			
	'06	'07	'08	'09
Solution	-11%	-28%	-29%	-61%
Foam		335%	23%	7%
Other Solution				171%
TOTAL	**13%**	**48%**	**10%**	**0%**

Source: nielsen

APPENDIX B

A multicenter, randomized, placebo-controlled, double-blind clinical trial of a novel formulation of 5% minoxidil topical foam versus placebo in the treatment of androgenetic alopecia in men

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Durham, North Carolina; Dallas, Texas; Cleveland, Ohio; Hershey, Pennsylvania; Minneapolis, Minnesota; and Morris Plains, New Jersey

Background: An alternative to currently marketed topical minoxidil solutions is desirable.

Objective: To assess the efficacy and safety of a new 5% minoxidil topical formulation in a propylene glycol–free foam vehicle in men with androgenetic alopecia (AGA).

Methods: This was a 16-week, double-blind, placebo-controlled trial of 5% minoxidil topical foam (MTF) in 352 men, 18 to 49 years old. At week 16, 143 subjects continued on an open-label phase to collect 52 weeks of safety information on 5% MTF.

Results: At week 16 compared with baseline, there was a statistically significant increase in (1) hair counts in the 5% MTF group versus placebo ($P < .0001$) and (2) subjective assessment of improved hair loss condition ($P < .0001$) in the 5% MTF group versus placebo. The 5% MTF was well tolerated over a 52-week period.

Limitations: There was no collection of efficacy data beyond 16 weeks.

Conclusions: We believe that 5% MTF is a safe and effective treatment for men with AGA. (J Am Acad Dermatol 2007;57:767-74.)

Following the initial reports in the 1980s,[1,2] minoxidil topical solution (MTS) has been a proven mainstay of treatment for male pattern hair loss (MPHL) or androgenetic alopecia (AGA). Two percent MTS is Food and Drug Administration (FDA) approved for both men and women[3,4] with AGA, and 5% MTS is FDA approved for men with AGA. The vehicle in MTS consists of water, alcohol, and propylene glycol, the latter increasing in amount with the higher concentration of minoxidil in order to solubilize the minoxidil.

A foam vehicle for delivery of 5% minoxidil (MTF) was identified as an alternative to 5% minoxidil solution. The 5% MTF formulation is a patented, hydroalcoholic, propylene glycol–free formula that is thermolabile and designed to melt at body

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Study results were presented as a poster at the Fall Clinical Dermatology Conference in Las Vegas, Nevada, October 6-9, 2006 and were presented at the Intercontinental meeting of the Hair Research Societies in Vancouver, British Columbia, June 13-16, 2007.

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Abbreviations used:

AEs:	adverse events
AGA:	androgenetic alopecia
FDA:	Food and Drug Administration
GPR:	global photographic review
MPHL:	male pattern hair loss
MTF:	minoxidil topical foam
MTS:	minoxidil topical solution
OTC:	over the counter
PK:	pharmacokinetic
TAHC:	target area hair count

temperature. Two preclinical studies evaluated comparative efficacy of the foam and solution vehicles. In the hamster ear model for assessing follicular targeting, the 5% MTF showed increased uptake of minoxidil over the 5% MTS at both 1 and 2 hours of application.[5] A direct comparison of the efficacy of 5% MTF and 5% MTS was performed in the stumptailed macaque,[6] an animal model for AGA in humans.[7,8] Six macaques were treated topically with either water, 5% MTS or 5% MTF once daily for sequential 4-month trial periods with 3-month washout periods between treatment groups.[6] Change in target area hair weight between baseline and month 4 of each treatment period was the primary end point. The macaques had an increase in hair weight of 12.40 mg (8.23-26.00 mg) on 5% MTF compared with an increase in hair weight of 9.27 mg (4.96-17.53 mg) on 5% MTS.

Two human pharmacokinetic (PK) studies were completed.[9] One study compared 5% MTS to 5% MTF and showed that the systemic absorption of the 5% MTF with twice-daily application of 1 g (one-half capful) in men was about half of that observed with 5% MTS with twice-daily application of 1 cc (both 100 mg daily of minoxidil); this was evidenced by the area under the curve of serum minoxidil concentration and maximum serum minoxidil concentration. The second study was an exaggerated-use PK study in men that demonstrated that application of up to 3 g of the 5% MTF or 300 mg of minoxidil (3 times the recommended dose) twice daily resulted in systemic levels that were well below the 21 μg/mL threshold for cardiac-related events and, thus, within acceptable safety margins. Consumer use studies[9] showed that the minoxidil foam vehicle was rated significantly higher on several aesthetic attributes compared with minoxidil solution, including ease of application, lack of dripping, quick absorption and drying, and ability to fit easily into a daily routine.

A double-blind, placebo-controlled study with an open-label safety extension phase was conducted to assess the efficacy and safety of 5% minoxidil topical foam (MTF) in men with MPHL. The results of this study, upon which FDA granted approval for the over-the-counter (OTC) use of 5% MTF in January 2006, are presented herein.

METHODS

Subjects

Men aged 18 to 49 years with Hamilton-Norwood patterns IIIv, IV, or V MPHL who were otherwise in good health were enrolled at one of 14 sites in the United States. Subjects were excluded from study participation if they had known sensitivity to minoxidil. Subjects were also excluded if they had used (1) topical minoxidil or any other OTC or prescription medication for hair growth within the past 6 months; (2) 5α-reductase inhibitors, isotretinoin, radiation to the scalp, or chemotherapy within the past year; (3) botanicals/neutraceuticals for hair regrowth for the past 3 months; or (4) systemic steroids for more than 14 days within the past 2 months prior to enrollment in the study. Men with uncontrolled hypertension or a history of hypotension, any chronic active scalp condition other than AGA, any untreated cancer excluding basal cell carcinoma and squamous cell carcinoma of the nonscalp areas, a history of hair transplants, scalp reduction, or use of hair weaves also were excluded.

Subjects must have agreed to use the same shampoo and to maintain the same hair style, hair length, and hair color during the entire study and to refrain from cutting the scalp hair shorter than 1 inch in length.

Study design

This study was conducted in two phases—a 16-week, double-blind, placebo-controlled phase to evaluate the efficacy and safety of the 5% MTF and a subsequent open-label extension phase to collect 52 weeks of safety data with 5% MTF.

All subjects signed an institutional review board (IRB)–approved informed consent form before participation in the study. Eligible subjects were randomized in a ratio of 1:1 to receive either 5% MTF or placebo twice a day. A 60-g can of study drug was dispensed at baseline and monthly thereafter. Subjects were instructed to dispense one half capful (1 g) of the study drug onto their fingertips and then to apply this directly to the affected vertex balding scalp twice a day. This was to be done after any shampooing or use of a hair dryer, and the study drug was to be allowed to dry naturally. Styling aids were to be applied only after the study drug had dried.

Subjects returned to the study center for compliance and safety evaluations at weeks 1 and 4 and, subsequently, for safety and efficacy evaluations at weeks 8, 12, and 16. At the completion of the

16-week, double-blind phase of the study, subjects were asked to continue on the study using active drug in order to gather a total of 12 months of safety information on 5% MTF. Subjects remained blinded to the treatment (active or placebo) that they had received while on the double-blind portion of the study until month 8 of the extension phase. At that time, if subjects had been on 5% MTF during the initial 16 weeks, the study was discontinued; if they had been receiving placebo during the initial 16 weeks, they continued on the study for an additional 4 months. Those subjects who had been taking active drug for the first 16 study weeks returned to the study center at weeks 24, 32, 40, 48, and 52. Those subjects who had been receiving placebo for the first 16 study weeks returned to the study center at weeks 24, 32, 40, 48, 56, 64, and 68.

Efficacy evaluation

Target area hair counts. Target area hair counts (TAHC) were performed at baseline and weeks 8, 12, and 16. For the double-blind phase of the study, one of the two coprimary efficacy end points was the change in TAHC between baseline and week 16. The percentage change in TAHC between baseline and week 16, while not a primary endpoint, was also evaluated.

At baseline, a circular area on the anterior leading edge of the vertex balding scalp was chosen as the target area for hair counts. A permanent ink dot tattoo was placed for precise localization of the target area on subsequent evaluations. The hairs in an area slightly larger than the 1 cm^2 target area were clipped to 1 mm. A 35-mm Nikon camera equipped with a device that allowed it to rest on the scalp and fix the distance and lighting of the attached camera (Canfield Scientific, Inc, Fairfield, NJ) was used to take the macrophotographs of the target area. These macrophotographs were sent to Canfield Scientific for processing. The macrophotographs were enlarged to an 8 × 10 size (5.7× magnification), a clear acetate overlay was attached, and all visible (nonvellus) hairs were dot-mapped by a technician trained in the procedure and blinded as to subject, treatment, and time. Dot maps were then translated to hairs by image analysis and a nonvellus TAHC was produced (nonvellus hairs/cm^2).[10]

Subject assessment. The other coprimary end point was subject assessment of improvement. Subjects were asked to fill out a questionnaire at week 16 that rated their overall hair loss condition in the vertex region compared to baseline. They rated their perception of their hair loss condition compared to baseline using a 7-point scale where −3 = significantly worse, −2 = moderately worse, −1 = minimally worse, 0 = no change, +1 = minimally improved, +2 = moderately improved, and +3 = significantly improved. To facilitate answering the questionnaire, subjects were provided with standardized Polaroid photographs of the vertex scalp taken at baseline and week 16. For each Polaroid photograph, the subject sat on a stool with the height fixed and placed his head in the stereotactic photographic device to ensure standardization of camera angle, head position, and lighting. Before taking the photographs, the hair in the vertex region was combed radially away from the center to maximize exposure of the hair loss. An attempt was made at the week 16 visit to duplicate the hair combing at baseline in order to facilitate direct comparison between time points.

Global photographic review. Global photographic review (GPR), also called expert panel review or global photographic assessment,[11] was a secondary end point. At baseline and weeks 8, 12, and 16, global photographs of the vertex scalp were taken with a 35-mm camera with the same protocol as noted above for Polaroid photographs. The 35-mm slides of baseline and one other study time point were then shown in a side-by-side presentation independently, and in a blinded fashion, to each of 3 experienced global photographic reviewers (Drs Olsen, Whiting, and R. Savin, MD). Room lighting, distance from screen to assessor, and magnification of the projected images were standardized. The global photographic reviewer then assessed the patient's hair loss compared with baseline using the same 7-point scale as subjects. The 3 GPR ratings were then compared. When two ratings were in agreement, the majority score was taken. If all 3 scores were different, the median score was taken.

Safety evaluation

Subjects were assessed at weeks 1, 4, 8, 12, and 16 for any intercurrent events and their potential relatedness to study drug as well as any symptoms of scalp irritation (stinging, burning, itching)—rated by the subjects as none, mild, moderate, or severe. Vital signs and visual assessment of the scalp for any dermatitis (erythema, dryness/scaling, and folliculitis) were rated by the investigator as none, mild, moderate, or severe. The returned container of study drug was weighed at each visit to determine the average dose. A complete blood cell count and serum chemistries and a urinalysis were performed at baseline and at weeks 8 and 16. If there was an event of any cardiac nature at any time point in the study (including a change in blood pressure, pulse, body weight, or hypertrichosis), investigators were instructed to draw blood for a serum minoxidil level.

Table I. Subject demographics by treat group—intent-to-treat population

Demographics	Treatment group	
	Placebo	5% MTF
Age, y		
No.	172	180
Range (min-max)	20.0-49.0	21.0-49.0
Mean (SD)	38.3 (±7.34)	40.1 (±6.33)
Race, No. (%)		
White	154 (89.5%)	151 (83.9%)
Black	5 (2.9%)	7 (3.9%)
Hispanic	7 (4.10%)	17 (9.4%)
Asian or Pacific Islander	3 (1.7%)	3 (1.7%)
American Indian or Alaskan	2 (1.2%)	2 (1.1%)
Other	1 (<1%)	—
Duration of hair loss (mo)		
No.	172	180
Mean (SD)	105.9 (67.03)	115.4 (77.03)
Median	96.0	108.0
Range (min-max)	5.0-312.0	12.0-336.0
MPHL No.(%)		
Type IIIv	63 (36.6)	77 (42.8)
Type IV	64 (37.2)	53 (29.4)
Type V	45 (26.2)	50 (27.8)
TAHC		
Mean (SD)	168.9 (48.45)	170.8 (50.4)
Median	167.5	167
Range (min-max)	69.0-324.0	79.0-329.0

MPHL, Male pattern hair loss; *MTF*, minoxidil topical foam; *SD*, standard deviation; *TAHC*, target area hair count.

In those subjects participating in the extension study, monitoring for adverse events (AEs) and vital signs was conducted at each visit. Repeat blood tests, urinalysis, and scalp assessments for any irritation (erythema, dryness/scaling, and/or folliculitis) were completed at the final visit of the open-label extension phase.

Statistical analysis

All efficacy and safety analyses were based on the intent-to-treat population. The intent-to-treat population included all randomized subjects.

Change in hair count was analyzed using analysis of covariance at each time point. If a subject's hair count data were not available, the last observation was carried forward. The analysis model included the treatment and center as factors and the subject's age as covariate. The mean difference of change in hair count and the 95% confidence interval of the mean difference were estimated from the model. The normality assumption of the analysis of covariance model was checked using the Shapiro-Wilk test, based on the residuals from the model.

Subject assessment of hair loss condition and the GPR score were analyzed in a way similar to that used for change in hair count, except there was no last observation carried forward since these subject assessments were collected only once at week 16.

Descriptive analysis was performed for the safety parameters.

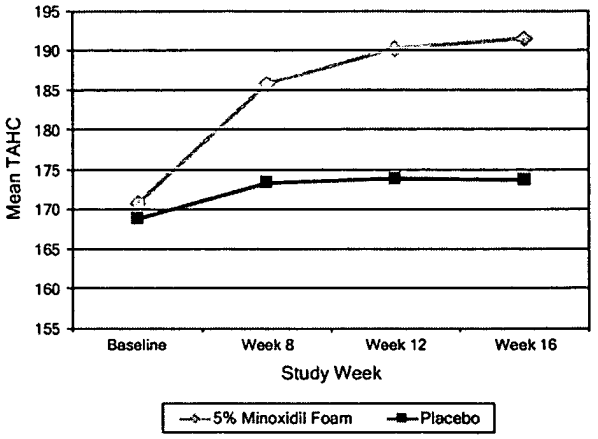

Fig 1. Mean target area hair counts—intent-to-treat population.

RESULTS

Baseline characteristics

A total of 352 male subjects between the ages of 20 and 49 years with MPHL were enrolled in the study; 172 were assigned to placebo and 180 to 5% MTF (Table I). The mean age of enrolled subjects was 39.2 years old, and the majority of subjects were Caucasian (86.6%). Forty percent of subjects had type IIIv, 33% had type IV, and 27% had type V Hamilton-Norwood hair loss pattern.

Subjects completing study

Three hundred fifteen of the 352 subjects completed 16 study weeks, of which 151 subjects were receiving placebo and 164 subjects were using 5% MTF. The reason for not completing the entire 16 weeks included withdrawn consent (8.1% on placebo, 4.4% on 5% MTF), lost to follow-up (2.3% on placebo, 2.8% on 5% MTF), and a nonserious AE (1.2% on placebo, 1.7% on 5% MTF). Of the 315 subjects completing the 16-week, double-blind phase, 143 entered the extension phase of the study. One hundred fourteen subjects completed 52 weeks on active drug, including 43% of subjects initially randomized to placebo and 57% of subjects initially randomized to 5% MTF. The reason for not completing the entire open-label period included withdrawn consent (11.8% on placebo, 4.0% on 5% MTF), lost to follow-up (8.8% on placebo, 6.7% on 5% MTF), a serious AE (1.5% on placebo, 0% on 5% MTF), and a nonserious AE (2.9% on placebo, 1.3% on 5% MTF).

Table II. Week 16 change from baseline hair count*

	Placebo			5% MTF			Total		
	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
Overall	156	4.7	19.7	167	20.9	22.5	323	13.1	22.6
Age group (y)									
18-25	11	14.3	17.6	3	22.7	6.7	14	16.1	16.0
26-30	13	7.3	18.3	13	27.1	19.4	26	17.2	21.0
31-35	28	4.9	21.5	19	20.7	17.9	47	11.3	21.4
36-40	33	1.6	21.9	43	18.9	28.1	76	11.4	26.8
>40	71	4.2	18.4	89	20.9	21.2	160	13.5	21.6
Hamilton-Norwood pattern									
Type IIIv	55	8.3	20.0	70	23.6	18.9	125	16.9	20.8
Type IV	62	4.2	17.7	49	15.9	25.7	111	9.3	22.3
Type V	39	0.6	21.7	48	22.0	23.4	87	12.4	24.9
Race									
White	140	4.6	19.9	140	21.5	22.9	280	13.1	23.0
Nonwhite	16	5.8	18.6	27	17.6	20.0	43	13.2	20.1
Duration of hair loss (y)									
<5	40	5.9	19.9	39	20.9	24.8	79	13.3	23.5
5-10	71	8.3	20.3	76	22.2	21.3	147	15.5	21.9
>10	45	−1.8	17.1	52	18.9	22.6	97	9.3	22.6

*Intent-to-treat population and last observation carried forward.

Compliance

Subject compliance was assessed from use of study drug. The mean number of days subjects were exposed to study medication and the actual/estimated daily study drug use were similar for the active and placebo groups. Mean amount of study drug used per day for each group was 2.2 g.

Efficacy

TAHC. There was a steady increase in TAHCs over the 16-week, double-blind phase in subjects on the 5% MTF (Fig 1). The mean change in TAHC at weeks 8, 12, and 16 was significantly greater for the 5% MTF group as compared to placebo at all time points (15.5 vs 5.2, 19.8 vs 5.0, and 20.9 vs 4.7 TAHC, respectively, $P < .0001$ for each) (Fig 1). Overall, following 16 weeks on 5% MTF, there was a mean 13.4% increase in TAHC over baseline, whereas the placebo group showed a 3.4% increase. As shown in Table II, the response to MTF was not affected by age, Hamilton-Norwood hair loss pattern, race, or duration of hair loss.

Subject assessment. There was a statistically significant difference between 5% MTF and placebo ($P < .0001$) for subject assessment of improvement of hair loss condition. Of subjects on 5% MTF, 70.6% felt their hair loss had improved from baseline and only 6.2% felt that it had worsened (Table III). In comparison, 42.4% of subjects on placebo felt their hair loss had improved from baseline, and 19.2% of subjects felt their hair loss had worsened. The subject assessment difference was more striking for the subjects who felt they had moderate or marked hair growth: 47.8% on 5% MTF vs 21.5% on placebo (Table III). The subject's age, Hamilton-Norwood hair loss pattern, race, or duration of hair loss did not affect the subject results.

Table III. Summary of efficacy at week 16*

Subject assessment of hair loss condition	Placebo (n = 172) No. (%)	5% MTF (n = 180) No. (%)
−3: Significantly worse	0	0
−2: Moderately worse	8 (4.7)	1 (0.6)
−1: Slightly worse	25 (14.5)	10 (5.6)
0: No change	56 (32.6)	32 (17.8)
+1: Slightly improved	36 (20.9)	41 (22.8)
+2: Moderately improved	28 (16.3)	47 (26.1)
+3: Significantly improved	9 (5.2)	39 (21.7)
Data not available	10 (5.8)	10 (5.6)

*Intent-to-treat population.

GPR. In the blinded GPR by the expert panel of investigators, there was a statistically significant difference between 5% MTF and placebo ($P < .0001$). At week 16, 38.3% of subjects on 5% MTF were rated as having increased hair growth compared to 5.2% on placebo. The percentage of subjects who were rated as having moderate or marked growth was 7.8% on 5% MTF versus 0.6% on placebo (Table IV). Representative photographs are shown in Figs 2 and 3.

Safety

In the double-blind phase of the study, the overall incidence of AEs was similar in the placebo and

Table IV. Frequency of global photographic review scores at week 16 compared to baseline by treatment group*

GPR score for hair loss	Placebo (n = 172) No. (%)	5% MTF (n = 180) No. (%)
−3 = Greatly decreased	0	0
−2 = Moderately decreased	0	0
−1 = Minimally decreased	4 (2.3)	0
0 = No change	134 (77.9)	94 (52.2)
+1 = Minimally increased	8 (4.7)	55 (30.6)
+2 = Moderately increased	1 (0.6)	14 (7.8)
+3 = Greatly increased	0	0
Data not available	25 (14.5)	17 (9.4)

GPR, Global photographic review.
*Intent-to-treat population.

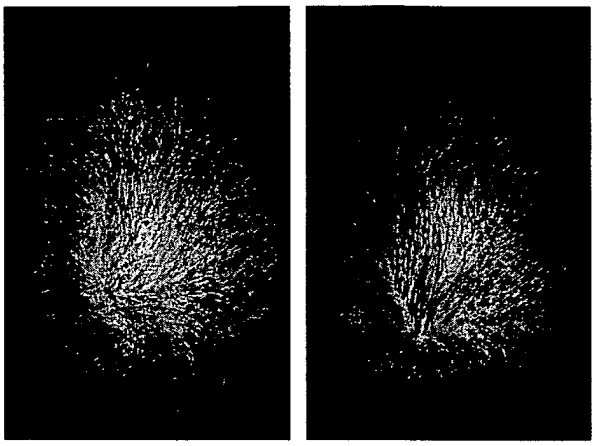

Fig 2. Subject, 41 years old, with Hamilton-Norwood type V MPHL, rated as having moderate hair growth by expert panel at week 16 on 5% MTF.

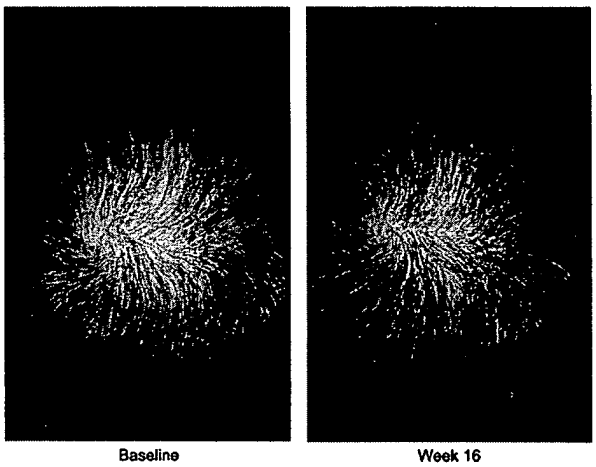

Fig 3. Subject, 33 years old, with Hamilton-Norwood type IV MPHL, rated as having moderate hair growth by expert panel at week 16 on 5% MTF.

active groups (46.5% in the placebo group and 45.6% in the 5% MTF group). The incidence of potential drug-related AEs was 6.7% for placebo and 7% for active drug. Only headache (placebo 1.2%, 5% MTF 1.7%), pruritus (placebo 0%, 5% MTF 1.1%), rash (placebo 0%, 5 % MTF 1.1%), and pain (placebo 1.2%, 5% MTF <1%) occurred in more than 1% of subjects in either treatment group. Dryness/scaling, erythema, and/or folliculitis were surprisingly common at baseline (14.0 % of placebo-treated subjects and 14.4% of 5% MTF-treated subjects), and there was no significant worsening of any of these signs of irritation in these subjects in either treatment group during the study (Table V). Overall, at the end of the 16-week placebo-controlled study, only 8.1% of those on placebo and 7.2% of those on 5% MTF showed any signs of irritation. Symptoms of irritation (stinging, burning, itching) occurred in 2.3% of those on placebo compared with 5.6% of those on 5% MTF, with itching (1.2% of those on placebo vs 4.4% of those on 5% MTF) accounting for the majority of the difference in groups. Most signs and symptoms were mild and intermittent in nature and only 8 of 172 subjects on placebo and 5 of 180 subjects on 5% MTF had greater than moderate levels of irritation at any time during the double-blind phase of the study.

There was no significant change in the overall incidence of AEs in the open-label phase of the study compared with the double-blind phase. The incidence of potential drug-related AEs in the open-label phase of the study remained low, with no AE occurring in more than 3% of study subjects: headache (2.1%); hypertension (1.4%); photosensitivity, nausea, weight gain, paresthesia, acne, pruritus, and rash each occurring in fewer than 1% of subjects. At the conclusion of the open-label phase, the signs of irritation were not vastly different than those exhibited at baseline but were more than those shown at the end of the double-blind phase (15/143 or 10.5%). Symptoms of irritation remained low at 2.9% overall. Again, most signs and symptoms were mild and intermittent in nature.

There were no drug-related serious AEs reported in the double-blind or open-label phases of the study in the placebo or 5% MTF groups.

There were no clinically significant laboratory abnormalities in subjects either on placebo or on 5% MTF. Additionally, there was no pattern of clinically relevant changes in vital signs (blood pressure, pulse, or body weight) in either phase of the study in subjects using 5% MTF.

Two subjects on placebo and one subject on 5% MTF in the double-blind phase, and 3 subjects on 5%

Table V. Subjects with scalp irritation*

Study phase	Treatment group	Signs of scalp irritation (investigator assessed)				Symptoms of scalp irritation (subject assessed)			
		Dryness/ scaling No. (%)	Folliculitis No. (%)	Erythema No. (%)	Overall No. (%)	Burning No. (%)	Itching No. (%)	Stinging No. (%)	Overall No. (%)
Double-blind	*Baseline*								
	5% MTF (n = 180)	15 (8.3)	4 (2.2)	12 (6.7)	26 (14.4)	0	2 (1.1)	0	2 (1.1)
	Placebo (n = 172)	15 (8.7)	3 (1.7)	12 (7.0)	24 (14.0)	0	1 (0.6)	0	1 (0.6)
	Week 16								
	5% MTF (n = 180)	5 (2.8)	2 (1.1)	7 (3.9)	13 (7.2)	3 (1.7)	8 (4.4)	4 (2.2)	10 (5.6)
	Placebo (n = 172)	7 (4.1)	2 (1.2)	8 (4.7)	14 (8.1)	2 (1.2)	2 (1.2)	0	4 (2.3)
Open-label	*Weeks 52/68* (n = 143)	5 (3.5)	7 (4.9)	9 (6.3)	15 (10.5)	2 (1.4)	5 (3.5)	2 (1.4)	5 (3.5)

*Includes slight or moderate degree.

MTF in the open-label phase had blood drawn to determine serum minoxidil levels secondary to an increase in blood pressure and/or body weight. The serum minoxidil levels were 1.17, 1.12, 1.02, and 0.397 μg/mL in subjects on 5% MTF and less than 0.350 μg/mL in subjects on placebo. All levels are within the range of serum levels seen with MTS, consistent with PK study results and well below the 21-μg/mL threshold for cardiac-related events with minoxidil.

DISCUSSION

Delivery of topical medications into the scalp is challenging. To be effective, (1) a majority of the medication must be delivered to the scalp, and medication lost on the hair or surrounding skin must be minimized; (2) the drug must be readily released from the vehicle; and (3) the drug must penetrate either the epidermis/outer root sheath of the infundibulum and/or the follicular canal and the protective layers that surround the hair shaft. Moreover, to ensure compliance, the medication must be cosmetically acceptable, especially if it is to be used daily and long term. This means it should be quick to dry, nongreasy, and should not affect the integrity of the hair by making it dry or brittle. Ideally, the constituents of the vehicle should themselves be nonirritating and of low allergic potential.

Since 1997, 5% MTS has been available OTC. GPR documents hair growth in 54% to 62% of men with Hamilton-Norwood pattern IIIv, IV, and V after 48 weeks of 5% MTS.[12] However, the novel foam vehicle utilized in this study appears to offer certain advantages over the solution vehicle, including the absence of propylene glycol (a potential irritant), the ability to limit spread beyond the intended application site, and less time to dry after application. Its enhanced cosmetic acceptability may also increase compliance with treatment, increasing the overall results with topical minoxidil. The mean increase at 16 weeks in both absolute TAHC and the change in TAHC relative to baseline was statistically significant ($P < .001$) between 5% MTF and placebo (20.9 vs 4.7 nonvellus hairs and 13.4% vs 3% total nonvellus hairs, respectively). Subjects on 5% MTF noted a mean 70.6% increase in hair growth versus 42.4% of subjects on placebo.

The incidence of pruritus with 5% MTF was 1.1% versus 6% seen in a separate trial of 5% MTS.[12] Overall, the incidence of irritation seen at baseline actually decreased during the study with both the foam vehicle and 5% MTF.

We conclude that the new 5% MTF preparation is a safe and effective treatment for MPHL.

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APPENDIX C

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Expression profiling and cellular localization of genes associated with the hair cycle induced by wax depilation

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The hair cycle is a highly regulated process controlled by multiple factors. Systematic analysis of gene expression patterns in each stage of the hair cycle would provide information useful for understanding this complicated process. To identify genes associated with the hair cycle, we used DNA microarray hybridization to analyze sequential gene expression patterns in mouse skin following hair cycle synchronization by wax-depilation. mRNA levels in mouse skin at various times after depilation were compared with those prior to depilation (resting phase). Nine, 9, 7, 51, 79, and 11 genes showed differential expression levels greater than 5-fold at days 1, 3, 6, 13, 19 and 21, respectively. According to their expression patterns, up-regulated genes were categorized into 4 groups: early anagen, middle anagen, late anagen/early catagen and middle/late catagen, and processes that take place in each stage were evaluated. On the basis of the list, we identified twelve new components that are specifically expressed in the hair follicle. In anagen follicle, carbonic anhydrase 6, cytidine 5'-triphosphate synthase, cathepsin E, poly A binding protein/ cytoplasmic 1, eukaryotic translation initiation factor 5, cytokeratin 12, keratin associated protein 3-3, and four functionally unknown genes were identified. On the other hand, only one genes, MMP-11 was up-regulated in catagen stage. These signals were confirmed using in situ hybridization. The strategy used here allowed us to identify unknown genes or processes previously not suspected to have a role in hair biology. These analyses will contribute to elucidating mechanisms of hair cycle regulation and should lead to the identification of novel molecular targets for hair growth and/or depilation agents.

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Microarray analysis of hair inductive signals from dermal papilla cells

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Toward the effort of the human hair follicle regeneration by cellular grafting, we performed microarray analysis to define molecules prerequisite for maintaining inductive ability of hair follicles. Because simple comparison between intact (active) and passaged (inactive) cells resulted in the changes of too many genes, we chose an alternative approach using overnight culture of murine dermal papilla enriched fraction under either low ($5\text{-}9\times10^4/cm_2$) or high ($3\text{-}7\times10^5/cm_2$) cell density condition. The cells under high density culture gave significantly higher hair inductivity (nine out of 13 grafts) than the one under low density (two out of 11 grafts) assessed at four weeks after co-grafting with new born epidermal cells on nude mouse back skin. Microarray analysis with oligo DNA microarray (Agilent) between the mRNAs extracted from low and high cell density (n=5) showed that 13 genes were up-regulated and seven genes were down-regulated significantly (more than two-fold). In addition to known molecules important for hair growth such as ephrin, thrombospondin, Kruppel-like factor, and BMP-7, previously unexplored molecules such as fibromodullin, serping, TGFβi, and connective tissue growth factor (CTGF; down-regulated), were identified as potential hair inductive molecules. Most of these genes are related to cell adhesion and cell-ECM matrix interaction. Cellular localization of these genes in human hair follicles was examined by immunohistochemistry. Among them, most striking staining pattern was observed with CTGF. In telogen hair, significant anti-CTGF immunoreactivity was observed in dermal papilla cells and this signals disappeared in anagen DP cells, being consistent with the microarray and real-time PCR results. These results suggest CTGF may act as an inhibitory molecule to suppress hair inductive ability of dermal papilla cells in telogen phase.

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Molecular mechanisms for the rhino-like phenotype: a new allele of the mouse hairless gene

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Mutations in the mammalian hairless (Hr) gene have provided instructive models for further studies of Hr function. Here we present a novel, autosomal recessive mutation in Hr, designated as HrrhR, which arose spontaneously at Oak Ridge National Lab. HrrhR/HrrhR mice begin to lose their hair at 2-3 weeks of age in a patchy manner beginning with the ventral surface. Hair loss is complete by ~ 5 weeks of age, revealing significant skin wrinkling that becomes increasingly severe with age. Due to the striking similarity to other Hrrh mutants, we directly sequenced Hr cDNA from wild type and HrrhR/HrrhR animals. A nonsense mutation was found in exon 12. The mutant mRNA is predicted to produce a truncated protein lacking TF_JmjC domain. Transcript analysis with Northern blot and RT-PCR indicated normal levels of Hr transcript, suggesting that the mRNA is not subject to nonsense mediated decay. Western blot analysis indicated that Hr protein was produced. Histological analysis of dorsal skin showed that hair follicles (HF) appeared largely normal at day 7, but by day 10 began to dilate at the apical surface, with the infundibulum progressively transforming into utricles and later into dermal cysts in adult animals. Because Hr encodes a transcriptional corepressor, we used oligonucleotide microarrays to identify differentially expressed genes in response to the HrrhR mutation but prior to an overt histological change. The analysis of dorsal skin gene expression profiles from 7 day-old HrrhR/HrrhR vs. HrrhR/+ mice identified the genes with differential expression, including genes known to be important in epidermal differentiation as well as novel genes with no defined function in HF development. A subset of these genes are likely to be direct targets of Hr regulation in HF. Network and pathway analysis were applied to these genes and suggest potential roles of Hr in HF development and maintenance.

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Macrophage activation and differential *in vivo* cytokine expression in primary cicatricial alopecia

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Primary cicatricial alopecias (CA) are rare disorders with an unknown etiology, a variable course of disease and an unpredictable response to treatment. The basic pathogenic mechanism of primary cicatricial alopecia is thought to be hair follicle stem cell failure that results in progressive, permanent destruction of hair follicles. The aim of this study was to assess the role of macrophages in lymphocyte mediated cicatricial alopecias in early affected and unaffected scalp and to determine the cytokine profile present. Immunohistochemical staining of vertical sections of scalp tissue with CD68 revealed markedly higher numbers of macrophages around the hair follicle, in the perifollicular connective tissue sheath of patients with cicatricial alopecia than in normal healthy volunteers. CD68 immunostaining was also performed on horizontal sections of the isthmic region for a more localized confirmation of the vertical section findings. These data support the idea that deletion of the hair follicles is caused by a macrophage-driven attack on epithelial hair follicle stem cells in the bulge of the outer root sheath. To clarify the role of macrophages and to identify the *in vivo* cytokine micromilieu, we used semiquantitative RT-PCR to investigate the expression of various cytokines and chemokines. The isthmic region was isolated via serial horizontal sectioning and H&E confirmation. RNA extraction and RT-PCR were then performed with this isolated tissue for each of the 8 CA patients and 5 normal scalp patients. The mRNA expression levels of cytokines IL6 and IL12, small inducible chemokines SCYA2 (MCP1), SCYA3 (MIP1) and SCYA27 (RANTES), and the matrix metalloproteinase MMP9 were significantly higher in CA compared to normal tissue. The upregulation of these genes in CA, independently confirmed by microarray analysis, suggest that components of immune signaling cascades, such as adhesion receptors, cytokines and chemokines play a fundamental role in the pathogenesis of cicatricial alopecia.

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Finasteride, selective 5-reductase inhibitor, inhibited the testosterone or DHT-induced TGF-β1 and type I procollagen expression in cultured human scalp dermal fibroblasts

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Androgenetic alopecia (AGA) is a dihydrotestosterone (DHT)-mediated process, characterized by continuous miniaturization of androgen reactive hair follicles. The miniaturization of hair follicles was accompanied by perifollicular fibrosis of follicular units. To understand the cause and role of perifollicular fibrosis in AGA, we investigated the effects of testosterone, DHT and finasteride, 5a-reductase inhibitor, on the expression of type I procollagen in cultured human scalp dermal fibroblasts. Testosterone (10-9-10-7M) and DHT (10-9, 10-7M) treatment increased the expression of type I procollagen mRNA and protein in a dose-dependent manner. Finasteride (10-9-10-5M) treatment also increased the type I procollagen expression. However, interestingly, pretreatment of finasteride (10-7M) inhibited the testosterone or DHT-induced type I procollagen expression at mRNA (59.8% % and 29.5%, respectively) and protein (75.1% % and 87.4%, respectively) levels. We also demonstrated that testosterone and DHT treatment increased the expression TGF-β1 protein levels (182% and 176%, respectively) in the culture media of human scalp dermal fibroblasts. Pretreatment of finasteride inhibited the testosterone or DHT-induced the expression of TGF-β1 protein by an average of 69% and 76%, respectively. Our findings suggest that testosterone /DHT-induced TGF-β1 and type I procollagen expression may contribute to the development of perifollicular fibrosis in the AGA, and the inhibitory effects of finasteride on testosterone/DHT-induced procollagen expression may contribute to hair growth by finasteride in AGA.

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Uptake of minoxidil from a new foam formulation devoid of propylene glycol to hamster ear hair follicles

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Post-marketing studies show that the present topical minoxidil formulations are considered oily and in some cases there are reports of skin irritation. A major cause of the apparent inferior cosmetic properties and adverse effects of the current formulations on the skin is the rather high content of propylene glycol. Thus a more cosmetically acceptable minoxidil foam formulation, devoid of propylene glycol was developed. In order to test the availability of minoxidil to hair follicles hamster ears were treated with minoxidil 5% foam in comparison to the current minoxidil 5% solution (Rogaine® Extra Strength), which served as a positive control. The foam was liquefied by gentle heating to 40C and then 20 µl was withdrawn with a positive displacement syringe and spread on the ventral ear surfaces of a hamster, continuously and lightly anesthetized by controlled inhalation of isoflurane. After 1 to 2 hours, the animal was sacrificed and the ears removed and carefully dissected to isolate the sebaceous gland minoxidil content as an aqueous solution. Each sample was analyzed by HPLC with electrochemical detection against minoxidil as an external standard. After one hour of minoxidil treatment of the hamster ears the foam showed a sebaceous gland uptake of 5.9% of the total minoxidil, while the positive control showed an uptake of 2.0% of the total minoxidil. After 2 hours of treatment the uptake from the foam was 6.5% in one series of experiments and 4.1% in another series of experiments, while the uptake from the positive control was 1.2% only. Thus the delivered dose of minoxidil from the foam to the hamster ear sebaceous glands after one hour treatment was about three times higher than for the minoxidil 5% solution. After two hours of treatment the minoxidil delivery from the foam formulation increased to 3.4 to 5.4 higher than for the minoxidil 5% solution. It is concluded that the new minoxidil 5% foam formulation is delivering minoxidil more effectively to the sebaceous gland of the hamster ear than does the current minoxidil 5% solution.

APPENDIX D

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Functional characterization of class II PI3-kinase in epidermal differentiation via targeted gene disruption and RNA interference

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Phosphoinositide 3-kinase (PI3K) gene family members affect proliferation, apoptosis and differentiation in a variety of tissues. A role for the class II PI3K C2beta in epidermal homeostasis has been suggested by PI3K pharmacologic blockade, which abolishes differentiation, and by C2beta overexpression in vitro, which induces keratinocyte differentiation. To define the role of PI3K C2beta in epidermal differentiation, we performed 2 independent approaches to genetic loss-of-function. RNA interference and generation of knockout mice. In vitro, RNAi-mediated C2beta knockdown failed to exert major effects on calcium-induced differentiation or cell proliferation. Mice generated from embryonic stem cells in which the C2beta gene had been deleted by homologous recombination also failed to display abnormal differentiation. C2beta-null murine keratinocytes also underwent normal calcium-induced differentiation in vitro. Consistent with this, multiple transgenic mice lines expressing epidermis-targeted C2beta also failed to display any disruptions in epidermal homeostasis in vivo, suggesting that prior in vitro overexpression studies were not in the range of physiologic relevance. These data indicate that C2beta is dispensable for normal epidermal homeostasis and differentiation; however, potential redundancy with the other class II PI3K expressed in epidermis, C2alpha, cannot be excluded and current efforts are directed at achieving simultaneous loss of function of both isoforms.

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Resveratrol-mediated modulation of NFκB activity by SIRT1 in human epidermoid carcinoma (A431) cells

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Resveratrol is a naturally occurring polyphenolic phytoalexin found in grapes, peanuts, and berries, and is known to have anti-tumor activity. Resveratrol treatment (25-100mM) of A431 cells induces G1/S cell cycle arrest, associated with the down-regulation of cyclin D1 and cyclin-dependent kinases 4/6, accompanied by an increase in hypophosphorylated Rb. The transcriptional factor Nuclear Factor kappa B (NFκB) is an important regulator of cell proliferation, and aberrant sustained NFκB activity characterizes various cancers. Acetylation as well as deacetylation of NFκB modulate its activity; specifically, deacetylation of p65/RelA leads to loss of transcriptional activity by promoting IκB interaction with NFκB. Sirtuins (SIRT), the mammalian homologs of the yeast SIR2 family, are nicotinamide adenosine dinucleotide (NAD)-dependent nuclear histone deacetylases. SIR2 is known to be involved in transcriptional silencing, DNA damage responses, and lengthening of the lifespan of yeast. We tested whether resveratrol, a known pharmacological agonist of SIRT, modulates NFκB activity in A431 cells. Resveratrol treatment (100mM) blocked phosphorylation-dependent degradation of a NFκB inhibitor protein, IκB-α. However, it induced nuclear translocation of p50 and p65/RelA in these cells, and increased NFκB -associated DNA binding activity, as measured by electrophoretic mobility shift assay. Further studies showed that the resveratrol-induced NFκB DNA-binding protein complex contained only the p50 subunit, but neither p65/RelA, nor the other subunits, c-Rel, RelB, and p52. SIRT1 was found to interact directly with p65/RelA in resveratrol-treated, but not in untreated control cells, suggesting that SIRT1 may deacetylate and deactivate p65/RelA. These results suggest that resveratrol has multiple effects on the NFκB pathway resulting in decreased cell proliferation which could account for its efficacy as a cancer chemopreventive agent.

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Protein tyrosine kinase Pyk2 is expressed in human epidermal keratinocytes and activated by stress- and differentiation-inducing stimuli

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The protein tyrosine kinase Pyk2 is a calcium-regulated kinase and a member of focal adhesion protein tyrosine kinase family. Pyk2 is suggested to function as an integrator of multiple signaling pathways, and may play a key role in fundamental cellular events. Pyk2 expression, regulation and functions have not yet been characterized in skin. Thus, we sough to investigate the expression and subcellular localization of Pyk2 in human epidermis and in primary human keratinocytes, and to examine activation of Pyk2 by differentiation- and stress-inducing stimuli. We report that human keratinocytes contain robust levels of endogenous Pyk2 protein as measured by Western blot analysis. Pyk2 activation was assessed by measuring its phosphorylation on tyrosine 402, a major autophosphorylation site. We show that Pyk2 is activated in skin cells by treatment with regulators of keratinocyte differentiation, including TPA and calcium. Adenoviral-mediated overexpression of PKC isoforms δ and η also leads to Pyk2 activation. Interestingly, treatment of keratinocytes with H_2O_2 results in extremely rapid (within 1 min), potent, and prolonged (up to 4h) activation of Pyk2. Pyk2 distribution in human epidermis and in cultured cells was studied by immunofluorescence. Pyk2 immunoreactivity is detectable in all epidermal layers. Strikingly, we show that in normal human epidermis and in unstimulated cultured epidermal cells, Pyk2 is predominantly nuclear. Some punctate membrane staining and diffuse cytoplasmic staining are also observed. A small fraction of Pyk2 is constitutively tyrosine phosphorylated in epidermis and in skin cells grown under basal conditions. It is localized at the sites of focal contacts and at cell borders. In summary, our results show that Pyk2 is expressed in epidermal cells and might be involved in keratinocyte differentiation and stress response. Further studies to define the role of Pyk2 in regulation of keratinocyte function are underway.

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$CD4^+/CD25^+$ cells promote systemic alopecia areata and $CD4^+/CD25^+$ cells blockade disease onset in the C3H/HeJ mouse model

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The potential for leukocyte transfer and blockade of alopecia areata (AA) development was examined using the C3H/HeJ mouse model by subcutaneously injecting normal haired mice with magnetic bead-conjugated antibody-separated cell subsets from AA affected mice. 9/10 CD8+ cell injected mice exhibited hair loss limited to the injection site. In contrast, 3/10 (30%, P=0.03) CD4+ and 10/23 (43%, P=0.0007) CD4+/CD25- cell injected mice developed systemic AA while a combined injection of CD8+ and CD4+/CD25- cells promoted AA in 5/8 mice (63%, P<0.0001). Injection of CD4+/CD25+ cells blockaded systemic AA induction by CD4+/CD25- cells (0/10, 0%, P=0.0148). The lymph nodes and skin infiltrating leukocytes were examined by flow cytometry 20 weeks post cell injection. There was a strong upregulation of IL-6, TNFα, IL-12 and IFNγ expression in mice developing localized or systemic AA. Increases in co-stimulatory ligands CD40 and CD80, plus increased leukocyte apoptosis resistance and reduced CD95, CD95L, and CD120b expression, were associated with successful systemic AA induction. Mice receiving CD4+/CD25+ cells that did not develop AA displayed a very high expression of IL-10 suggesting that the prevention of AA development after the co-transfer of CD4+/CD25+ cells may rely on their capacity to infectiously induce regulatory IL-10 cytokine expression in CD4+ cells. The results indicate CD8+ cells may be the primary effectors of hair loss phenotype, but systemic disease expression is determined by CD4+/CD25- cells. In combination, CD4+/CD25- and CD8+ cells act synergistically suggesting CD4+/CD25- cells induce hair loss primarily via auto-reactive CD8+ cell stimulation. This pathogenic activity defines the CD4+/CD25- cell population as a key component of AA and a prime target for the development of therapeutic strategies. Boosting CD4+/CD25+ regulatory cell activity may be one method of counteracting CD4+/CD25- pathogenicity.

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Hair growth efficacy assessment of a new topical minoxidil foam formulation in the stumptail macaque

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The currently available topical minoxidil formulations for treating pattern hair loss contain a substantial proportion of propylene glycol, which makes these formulations appear oily and in some cases irritating to the patient's scalp skin. A more cosmetically accepted and user-friendly minoxidil 5% foam formulation devoid of propylene glycol was developed and tested for hair-growing efficacy. A comparison was made to the hair-growing efficacy obtained for a topical minoxidil 5% solution (positive control) in a cross-over design in six stumptail macaque monkeys. The animals were housed in an AAALAC-accredited facility. Each formulation was applied to the balding scalp of the animals by painting with a brush. A scalp target area of one square inch was located by permanent tattooing in order to find the identical area repeatedly over the course of the study. The foam is thermo labile and will melt in contact with the scalp skin. For the present study the foam was liquefied by warming to body temperature before dosing. The dosing was 250 µl once a day for four months and there was a wash-out period of three months. The efficacy endpoint was weight of hairs cut from the target area after every 4 weeks. The accumulated hair weight measure during the four months was used for comparisons. Any adverse effects arising from use of the new formulations were also assessed. The dosing accuracy of the 250 µl dose was followed during a 17-day period by weighing the brush before and after application and was found to be around 20% (CV%), which was considered an acceptable accuracy. The minoxidil 5% foam resulted in a mean hair weight over the four months of 12.40 mg compared to 9.27 mg for the positive control ($p<0.031$). The formulations did not produce any adverse effects. Body weights were maintained and scalp skin inspections showed normal appearance of the skin. It is concluded that the new minoxidil 5% foam formulation is at least as effective as the current 5% minoxidil solution in the stumptail macaque.

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Serum and glucocorticoid-inducible kinase-like kinase plays a critical role in hair cycle regulation and hair shaft differentiation

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Recently, we have reported that a point mutation in *serum and glucocorticoid-inducible kinase-like kinase* (*Sgkl*) gene is responsible for the severe hair coat defect in the YPC mouse. We performed *in situ* hybridization to *Sgkl* mRNA in wild type mouse skin, and then analysed morphological and molecular characteristics of the hair follicle in the YPC ($Sgkl^{ypc}/Sgkl^{ypc}$). In the wild type hair follicle, *Sgkl* mRNA was found to be restrictedly expressed in the inner root sheath (IRS) during early to late anagen, but the expression was gradually diminished in catagen. The detailed histological observation revealed that the YPC hair follicle regressed at postnatal day 7 (P7), and surprisingly that the YPC still showed altered hair cycling pattern characterized by drastically shortened anagen. In addition, immunohistochemical and ultrastructual analyses revealed that the YPC hair follicle completely lacks in hair medulla at P5. GATA-3, which is related to hair shaft and IRS differentiation, was normally expressed in the IRS of YPC, but nuclear accumulation of β-catenin, which plays a critical role in Wnt signaling, was reduced in YPC at P3 and P5. In conclusion, from the observation of YPC mouse ($Sgkl^{ypc}/Sgkl^{ypc}$), SGKL is considered to play a critical role in postnatal hair shaft differentiation and hair cycle. In addition, Wnt/β-catenin pathway is suggested to be closely related to SGKL function in the hair follicle.